REMARKS

The Office action mailed 25 July 2006, has been received and its contents carefully noted. The pending, claims are 29-36, 38 and 39, were rejected. Claims 31-34 and 36 were withdrawn. By this amendment, claims 32 and 38 have been amended. Support may be found in the specification and the claims as originally filed, for example, page 28, lines 16-28. No statutory new matter has been added. Therefore, reconsideration and entry of the claims as amended are respectfully requested.

Objection to the Specification

The Examiner objected to the Title for not being descriptive and the Specification for improper trademark designations and use.

Applicants respectfully submit that the amendment to the Title and Specification obviates the objections.

Rejection under 35 U.S.C. 112, second paragraph

The Examiner rejected claim 38 under 35 U.S.C. 112, second paragraph, as being indefinite for reciting trademarks.

Applicants respectfully submit that the claim, as amended, obviates the rejection under 35 U.S.C. 112, second paragraph. Therefore, the rejection should properly be withdrawn.

The Claimed Invention

As provided in the parent application which issued as U.S. 6,746,850, the following summarizes the claimed invention:

The present invention is based on determining the "sensitivity coefficients" of given proteins. The meaning of and methods for obtaining the "sensitivity coefficients" are what distinguishes the present invention from prior art methods. A sensitivity coefficient is a constant that represents the amount or weight (fraction/percentage) a protein contributes to a reaction with a given substrate. Sensitivity coefficients may be determined in a variety of ways which include determining the reaction rates as a function of the sample concentration at infinite inhibitor concentration, plotting the best fit linear graphs, determining the slopes of the linear graphs and

then using simple subtraction to calculate the amount each protein contributes to the reaction.

Example 3 in the specification provides a detailed protocol for determining sensitivity coefficients of AChE and BChE towards ACT, PTC, and BTC. Each titration was fitted to the following equation:

$$V_{obs} = \frac{(Vc - Vr)K_I}{K_I + [I]} + Vr \qquad (1)$$

Since a plurality of proteins may have overlapping specificities for given substrates, all observable signals represent the sum total action of the plurality of proteins and cannot in and of themselves be used for directly measuring the amount of a single protein belonging to the plurality of proteins. Thus, the present invention provides a device which determines the weighted contribution for each protein belonging to a plurality of proteins in their total action on the given substrates. The weighted contributions are the sensitivity coefficients.

The concentration or activity of a given protein belonging to a plurality of proteins for a given substrate may be determined by treating each protein as an algebraic unknown and each observable reaction with the substrate as a result. As long as there is at least as many observables/results as unknowns, then each unknown can be explicatively quantified using any number of elementary algebraic methods for solving systems/sets of equations such as linear substitution/combinations known in the art. The only requirement is that the sensitivity coefficient for each unknown, i.e. the weight (fraction/percentage) that each unknown contributes to the overall observable reaction for the particular unknown need only be determined once.

It is important to note that the sensitivity coefficients may be obtained without adulterating the sensitivity coefficient sample by any number of methods including physically removing one of the unknowns, which usually alters the sensitivity coefficient sample and thereby changing the intrinsic contributions of each protein (sensitivity coefficients). Thus, a better alternative is to destroy the contribution of a given protein to the overall rate via a selective inhibitor. In this manner, it is possible to remove only the desired activity and leave the others intact. This is best accomplished via a titration curve (see, for example Figure A below) at a predetermined concentration of the sensitivity coefficient sample.
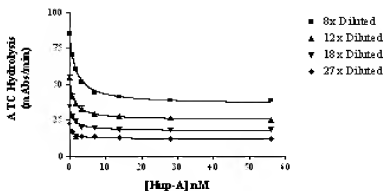
Figure A

So long as the predetermined concentration provides a signal that is interpretable over the entire range of the titration (i.e., 0 to infinite inhibitor concentration), then the asymptote represents the contribution of all other components of the system to the observable. The delta between the control value and the asymptote corresponds to the contribution that the selectively removed unknown contributed to the observable and is the (sensitivity) coefficient for that unknown at that sample concentration. If this is done as a function of sample concentration (Figure A), then the resulting plot displays how the sensitivity coefficients scale with sample concentration (see, for example, Figure B).
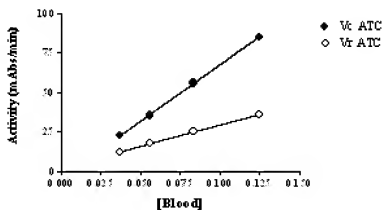

Figure B

Note that one needs to selectively remove each unknown component so that each sensitivity coefficient can be uniquely determined. It is possible to use inferred values based on the total observable minus the uniquely determined sensitivity coefficients to obtain either the

background or a remaining unknown's sensitivity coefficient in cases where selective inhibitors are not available. Likewise, a combination of selective inhibitors can be used to destroy activity to determine the sensitivity coefficient (i.e., asymptote) for an unknown for which a selective inhibitor is unknown or unavailable. In any case, the coefficients should be substantially identical.

As previously mentioned, with the sensitivity coefficients in hand, it is possible to use standard algebraic methods to uniquely quantify the concentrations of unknown proteins belonging to a plurality of proteins. For example, the concentrations a first protein, fp, and a second protein, sp, may be determined using the following mathematical equations:

$$x_1[fp] + y_1[sp] = R_1 \qquad \text{Substrate } 1$$
$$x_2[fp] + y_2[sp] = R_2 \qquad \text{Substrate } 2$$
$$x_3[fp] + y_3[sp] = R_3 \qquad \text{Substrate } 3$$

$$[fp]_{1,2} = \frac{\begin{vmatrix} x_1 & R_1 \\ x_2 & R_2 \end{vmatrix}}{\begin{vmatrix} x_1 & y_1 \\ x_2 & y_2 \end{vmatrix}} \quad \text{and} \quad [fp]_{1,3} = \frac{\begin{vmatrix} x_1 & R_1 \\ x_3 & R_3 \end{vmatrix}}{\begin{vmatrix} x_1 & y_1 \\ x_3 & y_3 \end{vmatrix}} \quad \text{and} \quad [fp]_{2,3} = \frac{\begin{vmatrix} x_2 & R_2 \\ x_3 & R_3 \end{vmatrix}}{\begin{vmatrix} x_2 & y_2 \\ x_3 & y_3 \end{vmatrix}}$$

$$\therefore mean\,[fp] = \frac{[fp]_{1,2} + [fp]_{1,3} + [fp]_{2,3}}{3}$$

The [sp] is calculated in the same manner as the [fp].

As provided above, the rates of substrate hydrolysis are represented by R1, R2, and R3 and correspond to the turnover of substrate 1, substrate 2, and substrate 3, respectively. The [fp] and [sp] refer to the actual concentrations of fp and sp in the sample. Finally, the coefficients in each equation (i.e., x1, x2, x3, and y1, y2, y3) represent sensitivity coefficients and are the contribution that fp and sp independently contribute to the overall rate of hydrolysis of each substrate (R1, R2, R3). Simultaneously solving these three sets of degenerate equations provides three independent estimates for the concentrations of fp and sp. Therefore, determining the mean value and the standard deviation for these independently derived values provides the concentrations of each protein, fp and sp. Examples of such are provided in detail in Example 3, entitled "Sensitivity Coefficient Determination, Method 1", Example 4, entitled "Sensitivity

Coefficient: Method 2" and may also be visualized in Figures 10A and 10B. See pages 37-42, and the figure legend described on page 14.

Prior to the present invention, no one thought to assay proteins based on their "sensitivity coefficients" in accordance with the present invention. Thus, nowhere in the prior art are devices, methods and means which allow one to determine the "sensitivity coefficients" of proteins taught or suggested.

Rejection under 35 U.S.C. 102(b)

The Examiner rejected claims 29 and 35 under 35 U.S.C. 102(b) as being anticipated by Doretti et al. Specifically, the Examiner deemed that Doretti et al. discloses the claimed invention.

Applicants respectfully submit that Doretti et al. does not anticipate the claimed invention because the device (and method) of Doretti et al. (1) requires the use of *immobilized* proteins, (2) requires the addition of *choline oxidase*, (3) measures *H_2O_2* as the indicator of protein amount (4) measures the activity of each protein using only *one substrate* and *no inhibitor*, and (5) must *separately* assay each protein in a sample with different sensors. The device and method of Doretti et al. is based on *co-immobilizing one cholinesterase and choline oxidase on a membrane*.

In contrast, the device of the present invention (1) does not require the immobilization of protein, (2) does not require the addition of choline oxidase, (3) measures the *sensitivity coefficients* of proteins rather than the production of H_2O_2, (4) requires the use of *more than one substrate* (a plurality), and (5) may assay more than one protein in a sample with the same device. Since the present invention is based on sensitivity coefficients rather than the production of H_2O_2, the specific binding reagents (proteins) need not be isolated (immobilized) or separated out from a mixture of a plurality of specific binding reagents. This is clearly different from the device and assay of Doretti et al. as a protein which has overlapping activities with the protein of interest will be expected to also produce H_2O_2, thereby falsely increasing the measured amount of the protein of interest.

In addition, Applicants respectfully submit that the measured "sensitivities" of the cholinesterases as recited in Doretti et al. are not the same as the "sensitivity coefficients" as

claimed and defined in the instant invention. Specifically, the "sensitivities" of Doretti et al. are merely the relative amounts of binding or kinetic constants for different substrates. See page 8, second paragraph. On the other hand, the "sensitivity coefficients" of the present invention are the weighted contributions that a specific binding reagent reacts with a substrate in the presence of other specific binding reagents that also react with the same substrate which is determined in the presence of an inhibitor. Doretti et al. does not use any inhibitor and the calculations which are based on one protein per one sample tested.

Since Doretti et al. does not teach or suggest a device (or method) of assaying the amount of a protein based on its sensitivity coefficient, the claimed invention is novel. Therefore, the rejection under 35 U.S.C. 102(b) should properly be withdrawn.

Rejection under 35 U.S.C. 103(a)

The Examiner rejected claims 29, 30, 35, 38 and 39 under 35 U.S.C. 103(a) as being unpatentable over Doretti et al. in view of Magnotti et al. and further in view of Ellman et al. Specifically, the Examiner deemed that it would have been obvious to develop a handheld device with a biosensor according to Doretti et al. to monitor enzyme activity.

As provided above, Doretti et al. does not teach or suggest the claimed invention as Doretti et al. does not disclose assaying the amount of a protein based on its sensitivity coefficient in accordance with the present invention. Applicants respectfully submit that neither Magnotti et al. nor Ellman et al. alleviate the deficiencies of Doretti et al. Specifically, Magnotti al. merely discloses some reagents used to assay cholinesterases. Magnotti et al. does not teach or suggest assaying the amount of a protein based on its sensitivity coefficient in accordance with the present invention. Ellman et al. discloses a colorimetric assay for acetylcholinesterase activity which was adapted for use in a handheld device. Ellman et al. does not teach or suggest assaying the amount of a protein based on its sensitivity coefficient in accordance with the present invention.

Since none of the cited references, alone or in combination, teach or suggest each and every limitation of the claimed invention, a prima facie case of obviousness has not been established. Therefore, claimed invention is unobvious and the rejection under 35 U.S.C. 103(a) should properly be withdrawn.

Request for Interview

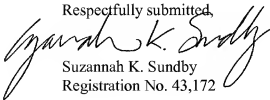Either a telephonic or an in-person interview is respectfully requested should there be any remaining issues.

As Applicants have found that the concept of "sensitivity coefficients" has been initially challenging for some of the Applicants' colleagues to understand, Applicants would greatly appreciate an in-person interview be granted prior to any adverse Office action such that prosecution on the merits may be advanced rather than being hindered by semantics and algebraic expressions.

CONCLUSION

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Therefore, it is respectfully requested that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, in the event that additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. 1.136(a), and any fees required therefor are hereby authorized to be charged to **Deposit Account No. 210-380**, Attorney Docket No. **034047.003DIV1 (WRAIR 00-23)**.

Respectfully submitted,

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